REMARKS

Claim Status

Claims 2-4 have been amended to remove the word "about". No new matter is introduced. With the present amendment, there are 49 claims pending, namely claims 1-49, among which claims 16-39, 42 and 43 have been withdrawn by the Examiner as being drawn to non-elected species and inventions.

Information Disclosure Statement

The Examiner indicates that the reference C8 in the Information Disclosure Statement (IDS) submitted October 27, 2003, is not being considered as it does not list a place and date of the publication. In response, Applicant submits a Supplemental Information Disclosure Statement to disclose the reference C8 with the place and date of the publication listed.

The Examiner further indicates that the reference C2 in the Supplemental Information Disclosure Statement (Supplemental IDS) submitted September 20, 2004, is not being considered as it is not a publication. In response, Applicant submits that C2 cites the International Search report (ISR) from the corresponding PCT application and that the individual references cited in the ISR were already included in either the IDS submitted October 27, 2003, or the Supplemental IDS submitted September 20, 2004. As such, citation of the reference C2 will not be amended.

Claim Interpretation

The Examiner states that the term "fluorophore compound" was not defined and that it is interpreted as either unimolecular or multimolecular entity. In response, Applicant submits that the instant specification clearly describes a fluorophore compound as a

unimolecular entity. In particular, paragraph [0032] describes "the molecule containing the fluorophore and the quenching leaving group can be any type of molecule. For example, the molecule can be an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, a carbohydrate, or other types of molecules." Also, Figures 2 and 3 of the instant specification provides examples of the fluorophore compound, which clearly demonstrate the relative locations of the fluorophore group and the fluorescence quenching leaving group on the same molecule as one entity.

The Examiner also states that the term "fluorescence quenching leaving group" was not defined and that it is interpreted as any fluorescence quenching group. In response, Applicant submits that the instant specification clearly sets the metes and bounds of this term and that not all fluorescence quenching groups are considered as fluorescence quenching leaving groups according to the context of the present application.

In particular, the instant specification provides the following definition to the term "leaving groups" in paragraph [0035]:

> "Leaving groups as in general are defined by (a) their ability to activate an atom (to which they are attached) for attack by a nucleophile group and (b) to leave (either simultaneously or subsequently) when the nucleophile does attack."

In addition, the leaving group itself is also a quencher in the context of the present application, such that when the bond is broken and the leaving group leaves, the fluorescence of the remaining molecule increases.

Furthermore, the instant specification provides multiple examples of fluorescence quencher (*see*, paragraphs [0041] and [0042].) The above description and examples indicate

that the fluorescence quenching leaving group in the context of the present invention is not any fluorescence quenching group; rather, it is a group that is both a quencher and a leaving group.

The Examiner further states that it is not defined what it means for the fluorescence to be quenched and that in the case of a fluorescence donor-acceptor pair, the acceptor is considered as a fluorescence quencher. In response, Applicant submits that the instant specification unambiguously describes what it means for fluorescence to be quenched.

In particular, the instant specification describes that nucleophilic attack on the quenched DNA causes release of the quencher group, which results in a ligated molecule that is now fluorescent due to the absence of the quencher group (*see*, paragraph [0019]), and that upon ligation with another molecule in intermolecular fashion, or with itself in intramolecular fashion, the quenching leaving group is displaced and the fluorophore is no longer quenched (*see*, paragraph [0031]). In addition, examples are given regarding quenched fluorescence. Example 2 of the instant specification describes that when beads containing a 7mer MUT probe autoligate a 13mer quenched electrophile probe to themselves, in the presence of the correct target DNA, the beads would become fluorescent, as the dabsylate group was lost and the nearby fluorescein label lost quenching. Such description and example indicate that quenched fluorescence in the context of the present invention involves a quenched electrophile probe, which is a unimolecular entity with both a flurophore group (e.g., fluorescein) and a fluorescence quenching leaving group (e.g., dabsylate group) located close to each other thereon.

In the case of fluorescence donor-acceptor pair, the fluorescence acceptor can be considered as a fluorescence quenching leaving group according to the context of the present

invention **only if** (emphasis added) the fluorescence acceptor is also a leaving group; and the fluorescence donor-acceptor pair can be considered as the fluorophore compound according to the context of the present application **only if** (emphasis added) both the fluorescence acceptor and the fluorescence donor exist on the same molecule (e.g., a probe).

Applicant further submits that acceptor dyes are well-known as fluorescence quenchers. In fact, one of the mechanisms by which dabsyl quenches fluorescence is by accepting energy from it. Some acceptors are non-fluorescent (e.g. dabsyl) and some are fluorescent (e.g. TAMRA). Either non-fluorescent or fluorescent acceptors can be considered as the fluorescence quenching leaving groups according to the context of the present invention as long as they quench by at least 2-fold and are also leaving groups.

The Examiner yet further states that the terms "about 2 fold", "about 100 fold" and "about 1000 fold" were not defined and that any value of quenching is considered as anticipating these terms. In response, Applicant submits that the term "about" has been removed from the related claims and that as such, the value of the quenching that is encompassed by the instant claims is clear and unambiguous.

Claim Rejection – 35 USC §112, Second Paragraph

Claims 2-4 are rejected under 35 USC §112, second paragraph, as allegedly being indefinite. Applicant respectfully traverses this rejection.

The Examiner states that the recitation of "at least about X fold" is vague and indefinite as the phrase "at least" is contraverted by the term "about". In response, Applicant has amended claims 2-4 by removing the term "about". As such, the present rejection should be withdrawn.

Claim Rejection – 35 USC §102

Claims 1-7, 9 and 11-15 are rejected under 35 USC §102(a) as allegedly being anticipated by Sando et al. (J. Am. Chem. Soc., vol. 124, pp. 2096-2097, February 2002). Applicant respectfully traverses this rejection.

Sando et al. describes the inventor's own work on which the instant invention was based. In addition, Sando et al. was published on February 13, 2002, which is within one year of the filing date (i.e., July 18, 2002) of the provisional application, of which the present application claims priority. As such, Sando et al. is not prior art. Accordingly, the present novelty rejection over Sando et al. should be withdrawn.

Claims 1-8, 10, 11 and 14 are rejected under 35 USC §102(a) as allegedly being anticipated by Livak et al. (PCR Meth. Appl., vol. 14, pp. 357-362, 1995). Applicant respectfully traverses this rejection.

Livak et al. teaches a quenched probe of an oligonucleotide with fluorescent dyes at opposite ends. In particular, the fluorescent dyes are a fluorescein reporter dye (e.g. 6-carboxyfluorescein (6-FAM) phosphoramidite) and a rhodamine quencher dye (e.g. 6-carboxytetramethylrhodamine succinimidyl ester (TAMRA NHS ester)). Livak et al. does not teach a fluorescence quenching leaving group, let alone a fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group, as claimed in the present application.

In detail, the rhodamine quencher dye taught by Livak et al. is not a leaving group. It is clear from Figure 1 on page 358 that the quencher dye of Livak et al. does not leave the oligonucleotide probe; rather, the fluorescein reporter dye leaves the probe when cleavage occurs between the fluorescein and rhodamine dyes. When the cleavage occurs, the

fluorescence intensity of the fluorescein dye increases because the fluorescein is no longer quenched. Also see the middle column, page 357 of Livak et al.

In view of the above remarks, Applicant submits that the oligonucleotide probe taught by Livak et al. is not the same as the fluorophore compound of the present application. As such, the present novelty rejection over Livak et al. should be withdrawn.

Claims 1, 5-7, 9 and 12-14 are rejected under 35 USC §102(a) as allegedly being anticipated by Xu et al. (Nat. Biotechnol., vol. 19, pp. 148-152, February 2001). Applicant respectfully traverses this rejection.

Xu et al. teaches two 7-mer probes (mutant and wild type) constructed with 3'-end phosphorothioate groups to act as nucleophiles in the ligation reaction and labeled with either the rhodamine (ROX) (for the mutant probe) or hexachlorofluorescein (HEX) (for the wild type probe), as well as one 13-mer probe (universal probe) internally labeled with 5-carboxyfluorescein (FAM). *See*, Sections titled "Design of autoligation probes" on page 148 and titled "Energy transfer probe design" on page 150. Xu et al. further teaches that FAM is the donor dye of fluorescence resonance energy transfer (FRET) and ROX or HEX is the acceptor dye of FRET. However, Xu et al. does not teach or suggest a fluorescence quenching leaving group for their 13-mer or 7-mer probe, let alone a fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group, as claimed in the present application.

In detail, FAM comprised in Xu's 13-mer probe is a fluorophore group that is considered to be the donor dye of FRET. Xu et al. is completely silent on any fluorescence quenching leaving group as part of their 13-mer probe. In fact, it is clear from Figure 3 that Xu's 13-mer probe does not comprise a fluorescence quenching leaving group. Whereas,

ROX or HEX comprised in their 7-mer probe, considered as the acceptor dye of FRET, is a fluorescence quencher but not a leaving group. In fact, no leaving group is suggested or demonstrated to be comprised in their 7-mer probe. *See*, Figure 3 of Xu et al.

In addition, as discussed above under the Section titled "Claim Interpretation", the fluorophore compound of the present application is a unimolecular entity. That is, the fluorophore group and fluorescence quenching leaving group are comprised in the same molecule as one entity. It is clear that neither the 13-mer nor the 7-mer probe taught by Xu et al. would be the same as the fluorophore compound of the present application for two reasons: 1) the fluorescein (i.e., FAM) and the fluorescence quencher (i.e., ROX or HEX) taught by Xu et al. are not comprised in the same probe as one entity; and 2) the florescence quencher ROX or HEX is not also a leaving group.

In view of the above remarks, Applicant submits that the 13-mer and 7-mer probes taught by Xu et al. are not the same as the fluorophore compound of the present application. As such, the present novelty rejection over Xu et al. should be withdrawn.

Claim Rejection – 35 USC §103

Claims 40, 41, 44, 46 and 47 are rejected under 35 USC §103(a) as allegedly being unpatentable over Sando et al., Tyagi et al. (U.S. Pat. No. 5,925,917) and Stratagene Catalog (page 39, 1988). Applicant respectfully traverses this rejection.

As discussed above, Sando et al. is not prior art.

Tyagi et al. teaches molecular beacons for allele discrimination. (*See* abstract.) In particular, molecular beacons are hairpin-shaped oligonucleotide probes that report the presence of specific nucleic acids in homogenous solutions. When they bind to their targets, molecular beacons undergo a conformational reorganization that restores the fluorescence of

an internally quenched fluorophore. The fluorophore becomes unquenched because the conformational reorganization of the molecular beacons (i.e., opening up of the hairpin) leads to the separation of the fluorophore from the quencher. *See*, Figure 1 of Tyagi et al. That is, the quencher does not leave the molecular beacons, and physically it remains part of the same molecule as the molecular beacons. As such, the quencher taught by Tyagi et al. is not the same as the fluorescence quenching leaving group of the present application. Therefore, Tyagi et al. does not teach or suggest a first nucleic acid probe comprising a fluorophore group and a fluorescence quenching leaving group, as claimed in the present application. In addition, Tyagi et al. is completely silent on a nucleophilic group-containing nucleic acid probe.

The excerpt of Stratagene Catalog discloses in general combining reagents into kit formats. Stratagene Catalog does not deal with fluorescent probes.

In view of the above remarks, Tyagi et al. in view of Stratagene Catalog would not have motivated one of ordinary skill in the art to produce a kit comprising first and second nucleic acid probes, wherein the first probe comprises a fluorophore group and a fluorescence quenching leaving group and the second probe comprises a nucleophilic group, as claimed in the present application. Rather, the combined teaching of Tyagi et al and Stratagene Catalog would, at most, lead to production of a kit comprising molecular beacons, which would be a different kit from that of the present application. As such, the present obviousness rejection should be withdrawn.

Claims 48 and 49 are rejected under 35 USC §103(a) as allegedly being unpatentable over Sando et al., Tyagi et al. and Stratagene Catalog as applied to claim 40 above, and

further in view of Seitz et al. (Angew. Chem. Int. Ed., vol. 39, pp. 3249-3252, 2000). Applicant respectfully traverses this rejection.

As discussed above, Sando et al. is not prior art. Tyagi et al. and excerpt of Stratagene Catalog, along or combined, do not teach or suggest a kit comprising first and second nucleic acid probes, wherein the first probe comprises a fluorophore group and a fluorescence quenching leaving group and the second probe comprises a nucleophilic group, as claimed in instant claim 40.

Seitz et al. teaches a doubly labeled peptide nucleic acid (PNA) probe having appropriately appended fluorescence donor and fluorescence quencher groups located close in proximity. *See*, Figure 1 of Seitz et al. When the probe sequence anneals to the target sequence, a structural reorganization increases the donor-quencher distance within the duplexes and fluorescence occurs. That is, the quencher does not leave the PNA probe of Seitz et al. Such PNA probe has a structural analogy to the molecular beacons of Tyagi et al. It is clear that Seitz et al. does not cure the deficiencies of Tyagi et al. and Stratagene Catalog. Even if one skilled in the art were motivated to combine the teachings of Tyagi et al., Stratagene Catalog and Seitz et al., he or she would not have produced a kit comprising first and second peptide nucleic acid (PNA) probes, wherein the first probe comprises a fluorophore group and a fluorescence quenching leaving group and the second probe comprises a nucleophilic group, as claimed in the present application (*see*, instant claims 40, 48 and 49). Rather, the combined teachings of Tyagi et al., Stratagene Catalog and Seitz et al. would, at most, lead to production of a kit comprising the molecular beacons of Tyagi et al. or the PNA probes of Seitz et al., which kit would be different from that of the present application. As such, the present obviousness rejection should be withdrawn.

Rejoining of Non-elected Groups and Species

Applicant respectfully requests that the method claims of Groups II and III be rejoined with the product claims of Group I that are currently under the examination if the Examiner finds the product claims are allowable upon considering the above amendments and remarks. *See*, MPEP §821.04.

Also, Applicant respectfully requests that the claims directed to the non-elected species be rejoined with the claims directed to the elected species for further examination if the Examiner finds the claims directed to the elected species are allowable upon considering the above amendments and remarks. *See*, MPEP 809.02(a).

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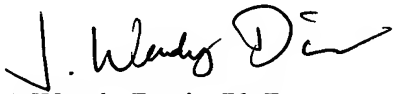
This response is filed along with a petition for a one-month extension of time. The Commissioner is authorized to deduct the extension fee ($60) from Howrey LLP Deposit Account No. 08-3038/12665.0024.NPUS01. Should any additional fees be required for any reasons relating to this document, the Commissioner is authorized to deduct said fees from the same Deposit Account.

Respectfully submitted,



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